Analysis of the May 24, 1999 Performance Evaluation Testing Results for Human T-lymphotropic Virus Types I and II Antibody Reported to the Centers for Disease Control and Prevention (CDC) by Participant Laboratories in the Model Performance Evaluation Program

This report is an analysis of results provided to the Centers for Disease Control and Prevention (CDC) by participant laboratories in the Model Performance Evaluation Program (MPEP) after they tested the human T-lymphotropic virus types I and II (HTLV-I/II) performance evaluation samples shipped to them on May 24, 1999. Testing results for this analysis were provided by 193 (84.3%) of 229 laboratories sent sample panels. Three laboratories returned result booklets with no testing results. Two laboratories returned results too late to allow their data to be included in this report. One laboratory used two different HIV-1 test kits to test the HTLV-I/II performance evaluation samples. The testing results reported by the participant laboratories reflect their testing performance using manufactured kits to test performance evaluation samples and do not necessarily reflect an evaluation of these manufactured kits.

Samples in this shipment consisted of plasma from donors who were HTLV-I/II antibody-negative (donor numbers 1 and 4) and donors antibody-positive for either HTLV-I (donor number 3) or HTLV-II (donor number 2). All laboratories participating in this survey received identical samples. Before shipment each donor sample was tested with two HTLV lysate-based enzyme immunoassay (EIA) kits licensed by the Food and Drug Administration (FDA) and with two HTLV Western blot (WB) kits. Additionally, each HTLV-I/II antibody-positive donor sample was tested by radioimmunoprecipitation assay (RIPA) and with an indirect immunofluorescent (IIF) antibody assay that can differentiate antibodies specific for HTLV-I or HTLV-II. Donor sample reactivity was determined by the CDC based on composite EIA, WB, and RIPA testing. The CDC MPEP interpretation of WB reactivity for each donor sample was consistent with the kit manufacturers' criteria for interpretation of WB results.

Figure 1 shows the cumulative frequency of test result interpretations reported by MPEP participant laboratories, arranged according to sample reactivity, for EIA, WB, and IIF methods. There were no false-positive or false-negative interpretations among the 1,143 EIA interpretations reported. One false positive and 11 indeterminate interpretations were among the 136 WB results reported. No false positive result, one false negative result, and 2 indeterminate results were reported using the Indirect Immunofluorescence test.

Results reported by two laboratories that correctly identified all HTLV-I/II antibody-negative and 3 of 6 HTLV-I/II antibody-positive samples using a particle agglutination test kit, Serodia-HTLV-I, manufactured by Fujirebio, Inc., are shown in figure 10. Also shown in this figure are results of a laboratory using an in house RIPA method which correctly identified one of three HTLV-I/II antibody-positive samples.

The types of laboratories that reported HTLV antibody testing results to CDC are shown in Figure 2. Each laboratory type is noted, by decreasing frequency, for each of the test methods.

The "Other" category includes, for example, research laboratories, organ procurement laboratories, drug screening/toxicology laboratories, and sexually transmitted diseases clinics.

The combinations of EIA, WB and IIF test methods used by laboratories and frequency of use are shown in Figure 3. Most laboratories performed only EIA (83.9%), while some laboratories performed both EIA and supplemental tests (14.5%). Three laboratories (1.6%) performed only supplemental tests (IIF or WB).

The types of test kits used, by manufacturer, for the EIA, WB, and IIF methods are shown, by decreasing frequency, in Figure 4. The Abbott HTLV-I/ HTLV-II EIA kit was used by 74.2% of laboratories reporting EIA results, the Organon Teknika HTLV-I/II kit was used by 18.9% of laboratories, and a variety of other test kits were used.

The results reported for the EIA, WB, and IIF methods, listed by kit manufacturer, for the CDC HTLV-I/II survey samples are shown in Figures 5, 6, and 7.

EIA Results

All results were correctly reported.

WB Results

A total of four indeterminate WB results were reported for the HTLV-II antibody-positive sample, CDC donor #2; two by a laboratory using a BioMerieux/Cambridge Biotech kit, and two by a laboratory using a Genelabs Diagnostics/Diagnostic Biotech kit. One false-positive result was reported for a HTLV-I/II antibody-negative sample, CDC donor # 4, by a laboratory using a BioMerieux/Cambridge Biotech kit. Seven indeterminate WB results were reported for CDC donor #1, a HTLV-I/II antibody-negative sample; 6 by laboratories using BioMerieux/Cambridge Biotech kits and 1 by a laboratory using a Genelabs Diagnostics/Diagnostic Biotech kit.

Of the 29 participant laboratories reporting WB results, 27 (93.1%) provided information regarding the criteria used for WB interpretations. Eighteen of these (66.7%) used WB interpretive criteria published by the manufacturer of the WB kit they used for testing. Other laboratories used the interpretive criteria published by the Association of State and Territorial Public Health Laboratory Directors (ASTPHLD; now known as the Association of Public Health Laboratories, APHL), 2 (7.4%); or "Other" criteria, 3 (11.1%). One laboratory indicated using the Public Health Service (PHS) Working Group criteria. Three laboratories indicated using the criteria of the World Health Organization (WHO). The criteria of these organizations are described in the following table:

CRITERIA FOR INTERPRETATION OF HTLV WESTERN BLOT TESTS

Organization	Criteria for Positive Test
Public Health Service (PHS) Working Group	p24 **and** gp46 or gp61/68
Association of State and Territorial Public Health Laboratory Directors (ASTPHLD, now Association of Public Health Laboratories or APHL)	p19 or p24 **and** one env* band
Consortium for Retrovirus Serology Standardization (CRSS)	p19 or p24 **and** gp46 or gp61/68
World Health Organization (WHO)	One gag** **and** one env band

*** env bands = gp21, gp46, gp61/68**
**** gag bands = p15, p19, and p24**

IIF Results

All interpretations by laboratories using IIF technologies were reported correctly for the HTLV- I/II antibody-negative samples. A false negative result was reported by a laboratory using a noncommercial source and 2 indeterminate results were reported by a laboratory using a procedure developed in house (Figure 7).

Western Blot Band Patterns

The percentage and frequency of WB protein bands reported are shown in Figure 8. The frequency of a reported band is shown above or within the column, and the number of reports is listed in the far right column. For the HTLV-I/II antibody-positive donor samples (donor numbers 2-3), the participating laboratories detected antibodies to most of the native viral-specific proteins (e.g., p19, p24, p32/33, and gp46). The presence of recombinant gp46-type I (r46I) was usually correctly reported for the HTLV-I antibody-positive sample (one laboratory reported a r46II band), and recombinant gp46-type II (r46II) was correctly reported for the HTLV-II antibody-positive sample by the laboratories using commercially available WB strips designed to detect these bands.

IIF Fluorescence Intensity

The fluorescence intensity patterns of HTLV-infected cells, as reported by participant laboratories, are shown in Figure 9. The number of reports received for each donor sample is listed in the far right column. Three laboratories reported IIF results. Some laboratories, however, reported only interpretations, and therefore, intensity data is not available for all donor samples. Generally, laboratories reported 2+ or greater fluorescence intensity in HTLV-infected cells for the HTLV-I/II antibody-positive samples for which intensity information was collected. One laboratory reported no fluorescence intensity for an HTLV-II antibody-positive sample,

donor number 2. No IIF reactivity was reported for any of the HTLV-I/II antibody-negative samples (Donors 1 and 4) tested.

Quality Control Testing

Although information was requested on the use of quality control (QC) materials not included with the manufacturer's kits, some laboratories continue to describe the kit controls as their only QC material. Positive and negative samples included in manufactured kits are internal kit control materials used to verify each lot's performance, calculate EIA test run cutoff values, and provide visual guidelines for determining band intensity for reading WB test results. They are often of limited value in assessing test performance over multiple lots of reagents. To verify the performance specifications of a test method and confirm that the accuracy and precision of a procedure are adequate, laboratories would benefit from testing external positive and negative QC samples, that is, samples which closely mimic patient specimens and are independent of the manufacturers' kit controls. An analysis of external control values over time allows a more accurate detection of shifts and trends in an analytic testing process resulting from testing problems such as faulty pipettors, inadequate incubation conditions, or kit lot sensitivity.

Of the 190 laboratories reporting EIA test results, 185 responded to the question whether they used external EIA QC samples. Of these, 116 (62.7%) indicated they used external EIA QC samples. Of the 116 laboratories using external EIA QC, 93 (80.2%) indicated they obtained QC samples for EIA testing from commercial sources. Forty-eight percent of the 116 laboratories used a single serum/plasma and 52% reported using multiple sera/plasma. Fifty-two percent (60 of 116) of the laboratories used a weakly positive external control. Fifty-seven percent (66 of 116) used external EIA QC with each set of EIA plates and 35% (41 of 116) used external EIA QC with each plate run.

Of the 29 laboratories reporting WB results, 11 (37.9%) reported the use of external QC samples in WB testing. Of the 11 laboratories using external WB QC samples, 9 (82%) indicated they obtained HTLV WB QC samples in house while 4 (36%) used a commercial source for WB QC samples (some laboratories used more than one source). Six (55%) of the 11 laboratories reported using external QC material with each set or run of WB strips.

One of three (33%) laboratories reporting IIF results reported using external QC samples.

Conclusion

Most of the laboratories participating in this survey correctly identified the HTLV-I/II antibody-positive and antibody-negative samples. Since no incorrect EIA interpretations were reported, the sensitivity, specificity and analytic performance for this test are 100%. If indeterminate interpretations are considered correct for antibody-positive samples, the sensitivity is 100%, the specificity is 83.3%, and the analytic performance is 94.1% for the WB test. Again considering indeterminate results as correct for antibody-positive samples, the sensitivity is 91.7%, the specificity is 100% and the analytic performance is 95.8% for the IIF test.